Research article

The aim of the measurement of Epstein-Barr virus DNA in hydroa vacciniforme and hypersensitivity to mosquito bites

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A running head: The meaning of EBV DNA load for HV and HMB.

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Abstract

Epstein-Barr virus (EBV) DNA load in the blood increases in post-transplant lymphoproliferative disorders and chronic active EBV infection. In this report, we analyzed the EBV DNA load in the peripheral blood mononuclear cells (PBMCs) and plasma of patients with hydroa vacciniforme (HV) and/or hypersensitivity to mosquito bites (HMB) to understand the clinical significance of EBV DNA load. All 30 patients showed high DNA loads in the PBMCs over the cut-off level. Of 16 plasma samples, extremely high in two samples obtained from patients with hemophagocytic lymphohistiocytosis (HLH). The amount of cell-free DNA in plasma were correlated to the serum levels of lactate dehydrogenase, and inversely correlated to platelet counts. These results indicate that the EBV DNA load in PBMCs can provide one of the diagnostic indicators for HV and HMB and marked elevation of cell-free EBV DNA in plasma might be related to cytolysis such as that observed in HLH.

Keywords: Epstein-Barr Virus DNA load; hydroa vaccniforme; hypersensitivity to mosquito bite; hemophagocytic lymphohistiocytosis.

Introduction

Epstein-Barr virus (EBV)-associated T/NK lymphoproliferative disorders (LPDs) include chronic active EBV infection (CAEBV), hydroa vaccniforme (HV) and hypersensitivity to mosquito bite (HMB).[1,2] HV was first reported as a benign photosensitivity disorder of childhood, characterized by vesiculopapules on sun-exposed areas. In contrast to this classic type of HV (classic HV: cHV), patients with a severe form of HV associated with edematous face, fever, liver damage, and serious hemophagocytic lymphohistiocytosis (HLH) have been reported mainly from the Eastern Asia and Central America under the name of systemic HV (sHV).[3,4] The patients of sHV was listed in the WHO classification as HV-like LPD. On the other hand, patients with HMB, or SMBA in the WHO classification [2], are characterized by skin ulcers and swelling at the sites of mosquito and other insect bites or injection sites for vaccinations, and frequently show fever and lymphadenopathy. [5-7] Patients with HMB are also encountered in the endemic areas similar to those of sHV/HV-like LPD and CAEBV. Unlike patients with cHV, patients with sHV, HMB or CAEBV show a progressive disease with often fatal outcomes. [4,8] Jeffrey I. Cohen *et all* reported that in the WHO classification, CAEBV is divided into two major forms: systemic CAEBV and cutaneous CAEBV; overlap occurs with some patients with cutaneous CAEBV exhibiting clinical and pathological evidence of systemic EBV infection. The two main forms of cutaneous CAEBV include HV-like LPD and severe mosquito bite allergy (SMBA). [1]

Patients with cHV and HMB usually have a dominant clone of EBV-infected γδT cells and NK cells, respectively.[9] Patients with sHV without HMB are classified into two groups: γδT-cell-dominant or αβT-cell-dominant types. Since the patients may have different subsets of EBV-infected T/NK cells in the blood, the clinical manifestations of HV, HMB and CAEBV often overlap with each other in the clinical course. [3]

The increase of EBV DNA load in the peripheral blood is closely related to the occurrence of EBV-associated LPDs in recipients with organ or hematopoietic stem cell transplant. [10.11] Such EBV-associated LPDs are mainly caused by EBV-infected B cells. However, little is known about the clinical significance of EBV DNA load in patients with EBV-associated T/NK LPDs such as HV and HMB. Previously, Kimura *et al.* and our group reported that the EBV DNA load in the peripheral blood showed no relation to the survival rate of the patients with CAEBV, HV or HMB. [4.8]

As reported previously, EBV is latently infected in the patient's peripheral blood mononuclear cells (PBMCs) in an episomal fashion in most cases, [12] and cell-free EBV DNA fragments are mainly detected in the plasma. [13-15] Therefore, the EBV DNA loads measured separately in the PBMCs and plasma may have different meanings in different disease conditions. For instance, the EBV DNA load in plasma is generally high in patients with EBV-related hemophagocytic lymphohistiocytosis (HLH) or infectious mononucleosis (IM). [16]

In the present study, we measured the EBV DNA load separately in PBMCs and plasma samples obtained from patients with HV and HMB, and compared the results with clinical manifestations such as the disease subtype and disease severity, and laboratory data including blood chemistry tests, the major subset of EBV-infected lymphocytes and its cell numbers.

1. Material and Method:

1.1. Patients

A total of 30 patients including 12 patients with cHV, 9 with sHV, 5 with HMB only, and 4 with HMB associated with HV-like eruptions were enrolled (Table 1). The control peripheral blood samples were obtained from 4 patients with IM and 24 healthy individuals. This study was approved by our ethical committee (the institutional review board of Okayama University Hospital) in accordance with the 1975 Declaration of Helsinki.

1.2. Real-time PCR

DNA was extracted from PBMCs of these patients at our hospital first visit or during their clinical courses using a QIAamp™ Blood Kit (Qiagen, Netherlands), and polymerase chain reaction (PCR) amplification was performed using QuantiTect™ Probe PCR (Qiagen, Netherlands) by a Roche light cycler (Roche, Switzerland). The PCR primers for this assay were selected in the *Bam*HI M region. We detected the EBV DNA loads in PBMCs and plasma using this method. [3,4,9,13]

1.3. Detection of cell-free EBV DNA with DNase treatment

Cell-free EBV DNA consists of encapsidated virions or naked genomes derived from dying cells. Naked viral DNA was degraded by DNase I, but the viral capsid protects encapsidated virions from DNase I-digestion. After incubation of plasma samples (200μL) with or without 25μL DNase I (Promega, Madison) for 1hr at 37°C, DNA was extracted with QIAamp DNA

mini Kit (Qiagen, Netherlands). The EBV DNA load in plasma was measured as described above. EBV virion levels in plasma were calculated by the ratio of DNase I-treated EBV DNA load to DNase I-untreated EBV DNA load. Our previous study confirmed that the cell-free EBV DNA in the plasma of 7/9 patients with HV and/or HMB (77.8 %) exclusively consisted of the naked form of EBV genomes sensitive to DNase treatment. [13]

1.4. Reverse transcriptase (RT)-PCR

RNA was extracted from the samples with TRIZOL^{TM} reagent (Gibco BRL, Gaithersburg, MD), and the cDNA was generated with random hexamer (Takara). The EBV reactivation marker, BZLF1 was amplified by RT-PCR, using BZLF1-specific outer primers as previously described. [17,18] The integrity of the RNA was checked by the parallel amplification of beta-2-microglobulin (β2-MG).

1.5. Statistical analysis

We analyzed the relation of the characteristic skin symptoms, such as vesiculo-papules, erythema and scale crusts, and the DNA load during each patient's clinical course. The DNA load at the first visit was evaluated in relation to the routine laboratory test results and outcomes of patients using SPSS Windows version 20.0 software. Fisher's test and the Mann-Whitney t-test were used to evaluate differences among groups (P <0.05). Relationships between the DNA load in PBMC and plasma, or the DNA load and routine laboratory tests were evaluated using Pearson's test.

2. Results

2.1. Cellular and cell-free EBV DNA load in patients with IM, HV and HMB

The majority of healthy adults are known to possess a small number of EBV-infected B cells in the peripheral blood. Thus, we measured EBV DNA loads in PBMCs (n=30) and plasma samples (n=21) obtained from healthy individuals. In these individuals, the EBV DNA load ranged from 0.5 to 108 copies/μg DNA in the PBMCs, and from 50 to 100 copies/mL in the plasma samples. The EBV DNA load in plasma (4/4 cases) were exclusively composed of cell-free EBV DNA fragments, sensitive to DNase-treatment. Based on these findings, we determined the cut-off values as follows: 120 copies/μg DNA (the mean+3SD) for PBMCs, and 130 copies/mL (the mean+3SD) for plasma samples.

Then, we measured EBV DNA loads in 30 samples of PBMCs and 16 plasma samples from patients with EBV-associated T/NK LPDs including HV and HMB. The EBV DNA loads in these PBMCs ranged from 446 to 140,000 copies/μg DNA (the mean: 28,486 copies/ μg DNA), all of which were higher than the cut-off value. The EBV DNA loads in the plasma samples ranged from 0 (detection limit) to 50,000 copies/mL (the mean: 8,446 copies/mL), and 3 of the 16 samples were below the cut-off value (Figure 1). Of the 16 plasma samples, two samples obtained from patients with active HLH showed extremely high levels of cell-free DNA (50,000 and 28,600 copies/mL, respectively) (Figure 1). There was no relation between PBMC and plasma levels of the patients with HV and HMB (r=0.21).

157 The EBV DNA load in PBMC or plasma showed no relation to the survival rate of the
158 patients with HV and HMB (Figure 2.). The 5 of 6 patients with sHV died. The main cause of
159 death was one patient from bowel bleeding, one from HLH, one from multiorgan failure,
160 which showed invasion of EBV positive T cells in the esophagus, gastrointestinal tract and
161 myocardium, and two from unknown. The 2 of 9 patients with HMB only and HMB+HV
162 died. The main cause of death was related to HLH in one HMB patient and was unknown in
163 one HMB+HV (Table 1).
164 In patients with IM characterized by primary infection of EBV to B cells (n=4), all patients
165 except one had higher levels of EBV DNA loads in the PBMCs than the cut-off value,
166 ranging from 13.3 to 2,680 copies/μg DNA, but the mean EBV DNA load in the PBMCs of
167 the IM group (mean: 1,783 copies/μg DNA) was much lower than that of the HV and/or
168 HMB group (mean: 28,486 copies/μg DNA) (Figure 1).
169 **Correlation of EBV DNA load to the disease subtype, severity, and other laboratory**
170 **data**
171 Of the 30 PBMC samples examined, the PBMC EBV DNA loads ranged from 770 to 73,000
172 copies/μg DNA (mean: 15,948) in patients with cHV, 446 to 101,000 copies/μg DNA
173 (mean: 37,736) in patients with sHV, and 1,900 to 140,000 copies/μg DNA (mean; 41,653)
174 in patients with HMB with or without HV. There was no correlation between each clinical
175 subtype, even though the dominant EBV-infected lymphocyte subsets and their numbers

varied. Our series of patients with cHV (patient 2 in Table 1) , who were in a stable disease condition, had high EBV DNA loads (73,000 copies/μg), comparable to those of other subtypes, sHV and HMB, which had more serious disease or fatal outcome.

When cellular EBV DNA loads were monitored in the same patient with HV-like lesions, there was no clear correlation to the severity of cutaneous manifestations. The expression of the EBV reactivation marker, BZLF1 mRNA, is not related to the DNA load in PBMC or plasma (Table 1). The cellular EBV DNA loads in the PBMCs (n=30) showed no correlation to the following laboratory test results: white blood cell (WBC) counts, lymphocyte numbers, hemoglobin (Hb), platelet (PLT) counts, lactate dehydrogenase (LD), aspartate aminotransferase (AST), alanine transaminase (ALT) (Figure 3), or lymphocyte numbers, atypical lymphocyte numbers, and ferritin (data not shown). However, there was a relation between the cellular EBV DNA loads in the PBMCs and soluble interleukin-2 receptor (sIL-2R) (r=0.74) (Figure 3). Therefore, the results of EBV DNA loads in the PBMCs did not differentiate the benign subtype from the serious subtypes or represent the severity of the clinical course.

In contrast, the amounts of cell-free EBV DNA in the plasma samples (n=16) were correlated to elevated levels of serum LD and ALT, respectively (r=0.90 and 0.66), and reversely correlated to the PLT counts (r=-0.68) (Figure 3). The two patients with extremely high levels of cell-free EBV DNA in the plasma (patients 15 and 26 in Table 1)

showed high levels of LD (764 and 575 IU/L), and AST (48 and 17 IU/L), and low PLT counts (3.1 and 8.4 x 10^4/μL).

2.2. Patients with extremely high levels of cell-free EBV DNA in the plasma

Table 1: patient 15 (the same patient as in Ref. 19)

A 7-year-old girl of sHV presented with an abrupt onset of fever of 38 ℃, discharge blood and aphtha. The blood counts revealed white blood cells at 20500/μL, hemoglobin (6.9 g/dl), Plt (28.5 x 10^4/μL), LD (204 IU/L), AST (22 IU/L), and ALT (12 IU/L). Her colon lesions showed many CD8-positive cells and EBER-positive cells. After 4 months, she presented with vesicle-papules and scarring on her leg. Her skin lesions were waxed and waned and were treated with steroid pulse, cyclophosphamide or cyclosporine.

After about 21 months, she had a high-grade fever, and her blood counts revealed white blood cells at 900/μL, thrombocytopenia at 3.1 x 10^4/μL, and an increased percentage (86.3%) of CD8 cells. The elevated serum levels of LD (764 IU/L), AST (48 IU/L), ALT (75 IU/L), sIL-2 receptor (4,500 U/mL) and ferritin (371.3 ng/mL), and the increase of nucleated cells (130000/ μ l) and blood phagocytosis in bone marrow test, together with thrombocytopenia, suggested the occurrence of HLH.

The EBV DNA loads in PBMCs and plasma revealed 80,000 copies/μg DNA and 50,000 copies/mL, respectively. She was diagnosed as having HLH associated with CAEBV, and was initially treated with methylprednisolone pulse (20mg/kg/day×3 day) and intravenous

214 immunoglobulin, followed by cord blood transplantation with a successful outcome (Table
215 2).

216 **Table 1: patient 26 (the same patient as in Ref. 20)**

217 A 15-year-old Japanese boy with CAEBV had a 12-year history of HMB. His complete blood
218 counts revealed a normal range of white and red blood cells, thrombocytopenia (8.4 x
219 10^4/μL), and an increased percentage (40%) of NK cells with a CD2+, CD3-, CD4-, CD8-,
220 CD16-, CD56+ phenotype. The elevated serum levels of LD (575 IU/L), AST (17 IU/L),
221 ALT (79 IU/L), sIL-2R (4,760 U/mL) and ferritin (268 ng/mL), together with
222 thrombocytopenia, suggested the occurrence of HLH. The patient was treated with a daily
223 dose of prednisolone, 10 mg, and the dosage was increased to 40 mg/d when systemic
224 symptoms, such as a high grade fever and splenomegaly, appeared. The cellular EBV DNA
225 load in the PBMCs was moderately elevated (1,900 copies/μg DNA), while the cell-free
226 EBV DNA in plasma had increased up to 28,600 copies/mL. A bone marrow transplant was
227 prepared from an HLA-matched donor because of disease progression, but unfortunately the
228 patient died of a serious attack of HLH (Table 2).
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Discussion

The present study showed that all 30 patients with HV and HMB had high EBV DNA loads in the PBMCs, well over the cut-off value (120 copies/μg DNA), while only 3 of 16 patients contained cell-free EBV DNA in the plasma at levels over the cut-off value (130 copies/ml). Our data indicate that the measurement of EBV DNA load in the PBMCs provides a reliable diagnostic indicator for HV and HMB, as compared with the detection of cell-free EBV DNA in plasma. This is the same result of the patients with CAEBV by Ito and Kimura *et al*. [21.22] But this is the opposite result of the previous observations in a largely immunocompromised and hospitalized cohort by Kanakry *et al*: [23] In their study, the cell-free EBV DNA in plasma performs better than the cellular EBV DNA load in PBMCs. The discrepancy must derive from differences in the host immunological conditions and the cell lineage with EBV infection: mainly B-cells in immunocompromised hosts, and T/NK cells in HV and HMB.

There was no correlation between the EBV DNA loads and the clinical features of HV and HMB, including the disease subtype, disease severity, prognosis, major EBV-infected lymphocyte subset, and cell numbers. But the monitoring of the cellular EBV DNA load in PBMCs does not necessarily provide useful information for disease progression and the occurrence of serious complications. The observation periods of some patients are relatively short, so we need more follow up times for prognosis.

Among 16 plasma samples from HV and HMB, two plasma samples revealed extremely

high levels of cell-free EBV DNA; these were obtained from patients complicated with HLH. Our results corresponded well with previous observations that patients with EBV-associated HLH or IM generally showed high EBV DNA loads in PBMCs and plasma. [24] Regarding the pathogenesis of IM and HLH, EBV-infected cells are induced to cell death by host immune responses or apoptosis, and cellular EBV DNA is released from the cells. [13-15] Our previous data showed that the cell-free EBV DNA in plasma exclusively consisted of naked EBV DNA derived from cytolysis in patients with HV and HMB.[13] Furthermore, Kawada *et al* showed that the plasma DNA load of the patients with CAEBV were significantly high during the active diseases. [25] Thus, we compared the amounts of cell-free EBV DNA with blood test results related to cytolysis. The results showed that the amount of cell-free DNA was correlated to the serum levels of LD, and inversely correlated to the PLT counts in our series (Figure 3.). These results were strongly affected by the two patients with HLH. We investigated the plasma of the patient 15, treated with or without DNase-treatment by using real-time PCR method. DNase-treatment resistant EBV DNA ratio (the DNA treated with DNase-treatment/ the DNA treated without DNase-treatment) in the plasma were 13.85% before HLH, and 0% during HLH. Therefore, increased levels of cell-free EBV DNA in the plasma might be related to cytolysis of EBV-infected cells and an alert for serious complications such as HLH.

Conclusion

The measurement of cellular EBV DNA load in PBMCs provides a diagnostic indicator for HV and HMB but does not reflect the clinical subtypes or outcomes. The amount of cell-free DNA in plasma is less useful as a diagnostic indicator, but its marked elevation might be related to serious complications such as HLH.

Abbreviations and acronyms

Epstein-Barr virus (EBV), lymphoproliferative disorders (LPDs), hydroa vaccniforme (HV), hypersensitivity to mosquito bite (HMB), peripheral blood mononuclear cell (PBMC), classical HV (cHV), systemic HV (sHV), hemophagocytic lymphohistiocytosis (HLH), chronic active EBV infection (CAEBV), infectious mononucleosis (IM), polymerase chain reaction (PCR), platelet (PLT), lactate dehydrogenase (LD), aspartate aminotransferase (AST) , alanine transaminase **(**ALT) , soluble interlukin-2 receptor (sIL2R), bone marrow transplantation (BMT)

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Figure legends.

Figrue 1. The EBV DNA load in PBMCs, but not in plasma, has a diagnostic value for HV and HMB.

All 30 HV/HMB patients showed significantly higher levels of DNA in the PBMCs than those of healthy individuals. Patients with sHV (□) and HMB (○) showed HLH.

Figure 2. The relation of outcome and EBV DNA load or the EBV DNA load in PBMC and plasma.

There were no correlations between the EBV DNA load and the patient's outcome or the EBV DNA load in PBMC and plasma (r=0.21).

Figure 3. The relations of EBV DNA loads in PBMCs and plasma and routine laboratory tests results.

The EBV DNA load in plasma showed a close correlation with PLT, LD and ALT, respectively (r= -0.68, 0.90 and 0.66).

Figure1-3 Abbreviation： HV: hydroa vaccniforme, HMB: hypersensitivity to mosquito bite, IM: infectious mononucleosis PBMC: peripheral blood mononuclear cell, HLH: hemophagocytic lymphohistiocytosis, WBC: white blood cell, Hgb: hemoglobin, Plt: platelet, LDH: lactate dehydrogenase, AST: aspartate aminotransferase, ALT: alanine transaminase, sIL2R: soluble interlukin-2 receptor.

Table legend.

Table 1. The list of 30 patients with HV and/or HMB.

402 **Table 2. These patients with sHV or HMB of HLH.**

403 These patients showed extremely high levels of EBV DNA load in the plasma during HLH.

Table 1.

patient No./ Sex/ Age at onset (y)	clinical subtype	follow up time (y)	WBC (/μl)	Ly/Aty (/μl)	Hgb (g/dl)	Plt (10⁴/μl)	LDH (IU/L)	AST/ALT (IU/L)	sIL2R (IU/L)	Ferritin (ng/mL)	outcome	EBVDNA load plasma/PBMC	BZLF mRNA	subset	gd (/μl)	ab (/μl)	NK (/μl)
1/f/5	cHV	10	5050	1398.9/0	12.5	20.6	193	17/9	ND	ND	A	ND/1100	-		ND	ND	ND
2/m/3	cHV	4	14490	7389.9/144.9	12.8	27.1	244	30/16	1010	18	A	ND/73000	-		ND	ND	ND
3/f/4	cHV	7	8200	3690/0	12.8	29.1	213	25/11	ND	ND	A	ND/11000	-		ND	ND	ND
4/m/1	cHV	2	7440	3742/0	11.8	36.4	257	34/12	ND	ND	A	2120/12000	-	γδ	409.2	1647.3	242
5/m/5	cHV	4	6980	2959/0	12.7	27.6	239	28/11	ND	ND	A	ND/1500	-	γδ	548.1	1428.5	228
6/m/5	cHV	5	7000	3350/0	13.6	36.2	393	22/17	ND	ND	A	ND/770	-		ND	ND	ND
7/m/4	cHV	10	5100	892.8/0	12	24.3	425	27/18	323	ND	A	1060/3610	-	γδ	154	1354	234
8/f/5	cHV	9	4900	2205/0	ND	30.6	255	28/15	ND	ND	A	ND/12000	-		ND	ND	ND
9/f/6	cHV	1	5420	ND/ND	12.5	17.1	218	47/49	ND	ND	A	ND/1000	-	γδ	ND	ND	ND
10/m/5	cHV	3	6410	3200/0	12	26.4	292	27/13	ND	ND	A	1275/29300	-	γδ	240	2030	356
11/f/17	cHV	3	4720	2200/0	14.9	19.5	161	20/13	ND	ND	A	0/ 36100	-	γδ	299	1335	173
12/f/8	cHV	4	6150	3198/0	13	25.7	229	53/51	ND	ND	A	1070/10000	-	γδ→NK	187.9	922.4	721.9
13/m/6	sHV	4	5700	3169.2/0	14.3	24.4	238	21/14	ND	ND	A	ND/46000	-		ND	ND	ND
14/f/17	sHV	7	3900	819/0	11.4	18.7	200	24/26	ND	115.1	A	ND/3500	-		ND	ND	ND
15※/f/5	sHV	6	1100	583/22	8.5	3.1	764	48/75	4500	371.3	A	50000/80000	-		ND	ND	ND
16/f/17	sHV	7	3940	1059.8/0	10.6	21.2	308	36/28	ND	ND	D	811/446	+		ND	ND	ND
17/f/25	sHV	9	12800	2560/0	12	29.1	189	30/35	ND	65.6	D	1460/101000	+	αβ	0	12416	3200
18/f/10	sHV	8	5800	2204/0	12.9	17.5	124	20/17	14.6	ND	D	800/35000	ND		ND	ND	ND
19/m/13	sHV	4	5170	ND/ND	13.9	22.4	200	17/9	ND	ND	A	0/3160	-	αβ	46	1506	66
20/f/68	sHV	14.1	NC	ND/ND	ND	ND	ND	ND/ND	ND	ND	D	0/6520	+	αβ	ND	ND	ND
21/m/74	sHV	4.5	6200	ND/ND	11.3	9.2	300	216/116	ND	ND	D	13000/64000	+	αβ	7.75	1444.6	511.5
22/m/6	HMB	5	3700	1258/37	12.4	5.1	318	30/22	108	ND	A	ND/5700	+	NK	ND	1157	1182
23/f/8	HMB	3	8800	7110/0	12.1	11.6	400	221/400	55	ND	A	ND/28000	-	NK	ND	ND	ND
24/m/6	HMB	3	4870	1821/0	12.2	18.2	251	26/10	ND	ND	A	368/5580	-	NK	70	597	931
25/m/5	HMB	3	5200	ND/ND	11.1	16.9	454	52/62	ND	ND	A	ND/140000	ND	NK	ND	ND	ND
26※/m/3	HMB	4	8380	ND/ND	15.2	8.4	575	17/79	4760	268	D	28600/1900	+	NK	ND	ND	1681
27/m/20	HMB+HV	14	4900	1900/0	15.3	19.6	166	21/19	ND	ND	D	1070/31700	+	NK	ND	ND	1681
28/f/8	HMB+HV	2	7780	4745.8/0	12	35.7	266	38/34	34.8	531	A	8160/21000	-	NK	ND	465	1788
29/f/4	HMB+HV	19	3310	1095/0	12.6	27.5	243	23/10	ND	ND	A	ND/78700	ND	NK	ND	409	726
30/f/2	HMB+HV	1	5600	ND/ND	12.7	23.8	386	130/151	ND	ND	A	ND/11000	ND	NK	ND	ND	ND

Abbreviation：f: female, m: male, cHV: classical hydroa vaccniforme, sHV: systemic hydroa vaccniforme, HMB: hypersensitivity to mosquito bite, CAEBV: chronic active Epstein-Barr virus infection, WBC: white blood cell, Ly: lymphocyte, Aty: atypical lymphocyte, Plt: platelet, LDH:

lactate dehydrogenase, AST: aspartate aminotransferase, ALT: alanine transaminase, sIL2R: soluble interlukin-2 receptor, y: years, Dead: D, Alive: A, ND: not done, undetectable=0 , HLH: hemophagocytic lymphohistiocytosis. Patient No 20: ref 26. Patient No 21: ref 27.

※: The two patients (patient 15: ref 20 and patient 26: ref 21) developed HLH.

Table 2.

	Patient 15. A sHV patient with HLH				Patient 26. A HMB patient with HLH		
	day-10	day0	day13	day29	day -11	day 0	day 163
AST (IU)	43	99	138	53	4	106	34
ALT (IU)	93	109	279	189	40	265	78
LDH (IU)	413	992	476	278	571	980	615
Plt × 10^3 (/μl)	166	24	57	285	116	38	78
Ferritin (IU)	ND	2368.5	ND	25.1	129.5	393.3	114.5
EBV DNA load (plasma) (copy/ml)	ND	500000	300	600	3680	28600	6590

Abbreviation : HMB: hypersensitivity to mosquito bite, sHV: systemic hydroa vaccniforme, HLH: hemophagocytic lymphohistiocytosis, AST: aspartate aminotransferase, ALT: alanine transaminase, LDH: lactate dehydrogenase, Plt: platelet, ND: not done, day0 : the occurrence of HLH.